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Published in:
JACC. Heart failure

DOI:
10.1016/j.jchf.2019.08.004

Publication date:
2020

JACC: HEART FAILURE VOL. 8, NO. 1, 2020

Mediators of the Effects of Canagliflozin on Heart Failure in Patients With Type 2 Diabetes

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ABSTRACT

OBJECTIVES The purpose of this study was to explore potential mediators of the effects of canagliflozin on heart failure in the CANVAS Program (CANagliflozin cardioVascular Assessment Study; NCT01032629 and CANagliflozin cardioVascular Assessment Study–Renal; NCT01989754).

BACKGROUND Canagliflozin reduced the risk of heart failure among patients with type 2 diabetes in the CANVAS Program. The mechanism of protection is uncertain.

METHODS The percentages of mediating effects of 19 biomarkers were determined by comparing the hazard ratios for the effect of randomized treatment from an unadjusted model and from a model adjusting for the biomarker of interest. Multivariable analyses were used to assess the joint effects of biomarkers that mediated most strongly in univariable analyses.

RESULTS Early changes after randomization in levels of 3 biomarkers (urinary albumin:creatinine ratio, serum bicarbonate, and serum urate) were identified as mediating the effect of canagliflozin on heart failure. Average post-randomization levels of 14 biomarkers (systolic blood pressure, low-density lipoprotein and high-density lipoprotein cholesterol, total cholesterol, urinary albumin:creatinine ratio, weight, body mass index, gamma glutamyltransferase, hematocrit, hemoglobin concentration, serum albumin, erythrocyte concentration, serum bicarbonate, and serum urate) were identified as significant mediators. Individually, the 3 biomarkers with the largest mediating effect were erythrocyte concentration (45%), hemoglobin concentration (43%), and serum urate (40%). In a parsimonious multivariable model, erythrocyte concentration, serum urate, and urinary albumin:creatinine ratio were the 3 biomarkers that maximized cumulative mediation (102%).

CONCLUSIONS A diverse set of potential mediators of the effect of canagliflozin on heart failure were identified. Some mediating effects were anticipated, whereas others were not. The mediators that were identified support existing and novel hypothesized mechanisms for the prevention of heart failure with sodium glucose cotransporter 2 inhibitors. (J Am Coll Cardiol HF 2020;8:57–66) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ISSN 2213-1779 https://doi.org/10.1016/j.jchf.2019.08.004

ABBREVIATIONS AND ACRONYMS

BMI = body mass index

CI = confidence interval

DBP = diastolic blood pressure

eGFR = estimated glomerular filtration rate

FPG = fasting plasma glucose

GGT = gamma glutamyltransferase

HbA_{1c} = hemoglobin A_{1c}

HDL-C = high-density lipoprotein cholesterol

HR = hazard ratio

LDL-C = low-density lipoprotein cholesterol

MDRD = Modification of Diet in Renal Disease

NHE3 = sodium hydrogen exchanger-3

SBP = systolic blood pressure

SGLT2 = sodium glucose cotransporter 2

TC = total cholesterol

TG = triglyceride

UACR = urinary albumin:creatinine ratio

Large-scale trials of sodium glucose cotransporter 2 (SGLT2) inhibitors have shown marked reductions in the risk of heart failure (1). In the CANVAS Program (CANagliflozin cardioVascular Assessment Study; NCT01032629 and CANagliflozin cardioVascular Assessment Study-Renal; NCT01989754), the hazard ratio (HR) for hospitalized heart failure was 0.67 (95% confidence interval [CI]: 0.52 to 0.87), in which effects appear early and are sustained during follow-up (2). The primary action of SGLT2 inhibitors is to prevent the kidneys from reabsorbing glucose (3), which results in improved glycemia, weight loss, lowered blood pressure, and reduced excretion of protein from the kidney (4). There are other effects, including natriuresis and reductions in body fluid volume, that may have early and sustained effects on the risk of heart failure (5).

To qualify as a potential mediator, a biomarker must be both affected by the drug under investigation and associated with the outcome of interest. However, all biomarkers that meet these criteria are not necessarily mediators of the effect of the drug because the criteria could also be seen for biomarkers that lie outside the mechanistic pathway (6). The investigation of mediators is further complicated by the potential for confounding of associations, interactions between mediators, different levels of precision with which potential mediators can be recorded, different ways in which drug effects on mediators can be measured, and the choice of other covariates to be included in the models (7).

A recent mediation analysis of the effects of the SGLT2 inhibitor empagliflozin suggested that markers of plasma volume were the most important mediator for effects on cardiovascular mortality, but possible mediating effects were also identified for fasting plasma glucose (FPG), urinary albumin:creatinine ratio (UACR), and uric acid (8). Some mediators that might have been anticipated to be important, such as blood pressure lowering, were not found to contribute to the observed benefit. By contrast, others that might not have been anticipated to be strong mediators of protection against cardiovascular death, such as hemoglobin, had apparently large effects.

The goal of the present analyses was to explore potential mediators of the beneficial effects of canagliflozin on heart failure in the CANVAS Program.

SEE PAGE 67

METHODS

The CANVAS Program integrated data from 2 randomized trials comparing the effects of canagliflozin with those of placebo. All participants provided written informed consent, and the trials were registered.

PARTICIPANTS. Participants were individuals with type 2 diabetes and an elevated cardiovascular risk (2). Patients were either 30 years of age or older with a

Boehringer Ingelheim, Durect, Eli Lilly, Gilead, GlaxoSmithKline, Janssen, Merck, Novartis, Novo Nordisk, Pfizer, Pharmalink, Relypsa, Retrophin, Roche, Sanofi, Servier, and Vitae. Dr. Figtree has received research support from the cofunded Australian National Health and Medical Research Council and Heart Foundation fellowship and Heart Research Australia; and has received compensation from Janssen for serving on the adjudication panel of the CANVAS Program. Dr. Heerspink has served as a consultant for AbbVie, Astellas, AstraZeneca, Boehringer Ingelheim, Fresenius, Gilead, Janssen, Merck, and Mitsubishi Tanabe; and has received research support from AbbVie, AstraZeneca, Boehringer Ingelheim, and Janssen. Dr. Mahaffey has received research grants/contracts from Afferent, Amgen, Apple, Inc., AstraZeneca, Cardiva Medical, Inc., Daiichi, Ferring, Google (Verily), Johnson & Johnson, Luitpold, Medtronic, Merck, National Institutes of Health (NIH), Novartis, Sanofi, St. Jude, and Tenax; and has served as a consultant for Abbott, Ablynx, AstraZeneca, Baim Institute, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Johnson & Johnson, Medscape, Mitsubishi, Myokardia, NIH, Novartis, Novo Nordisk, Portola, Radiometer, Regeneron, SmartMedics, Springer Publishing, and University of California, San Francisco (UCSF). Dr. de Zeeuw has served on advisory boards and/or as a speaker for Bayer, Boehringer Ingelheim, Fresenius, Mundipharma, and Mitsubishi Tanabe; and has served on steering committees for AbbVie and Janssen; and has served on data safety and monitoring committees for Bayer. Drs. Vercruysse and Shaw are employees of Janssen Research & Development. Dr. Matthews has received research support from Janssen; and has served on advisory boards and as a consultant for Novo Nordisk, Novartis, Eli Lilly, Sanofi, Janssen, and Servier; and has given lectures for Novo Nordisk, Servier, Sanofi, Eli Lilly, Novartis, Janssen, Mitsubishi Tanabe, and Aché Laboratories; and currently serves as president of the European Association for the Study of Diabetes (EASD). Dr. Neal has received research support from the Australian National Health and Medical Research Council principal research fellowship; and has served on advisory boards and/or as a consultant for Janssen and Merck Sharpe & Dohme, with any consultancy, honoraria, or travel support paid to his institution. The George Institute for Global Health holds multiple additional commercial contracts with a diverse range of entities.

Manuscript received May 1, 2019; revised manuscript received July 24, 2019, accepted August 6, 2019.

TABLE 1 Effects of Canagliflozin on Biomarkers That May Mediate the Effect of Canagliflozin on Heart Failure

	Mean ± SE at Baseline		Difference ± SE at Follow-Up*	
	Placebo	Canagliflozin	Early	Average
Glycemia				
HbA_{1c}, %	8.25 ± 0.01	8.25 ± 0.01	**−0.62 ± 0.02**	**−0.52 ± 0.02**
Vascular tone				
SBP, mm Hg	136.90 ± 0.24	136.44 ± 0.21	**−3.58 ± 0.26**	**−3.91 ± 0.19**
DBP, mm Hg	77.81 ± 0.15	77.62 ± 0.13	**−1.52 ± 0.15**	**−1.33 ± 0.11**
Pulse rate, beats/min	72.49 ± 0.16	72.64 ± 0.14	0.06 ± 0.16	−0.22 ± 0.12
Lipids, mmol/l				
LDL-C†	2.30 ± 0.01	2.29 ± 0.01	**0.09 ± 0.01**	**0.11 ± 0.01**
HDL-C†	1.18 ± 0.005	1.18 ± 0.004	**0.04 ± 0.004**	**0.05 ± 0.003**
TG†	2.03 ± 0.02	2.02 ± 0.02	**−0.06 ± 0.02**	0.02 ± 0.02
TC†	4.37 ± 0.02	4.35 ± 0.02	**0.11 ± 0.02**	**0.16 ± 0.01**
Renal				
UACR, mg/g	20.49 (315.41)‡	19.87 (288.54)‡	**0.83 (0.81, 0.85)§**	**0.81 (0.79, 0.83)§**
eGFR, ml/min/1.73 m^2	76.18 ± 0.32	76.68 ± 0.27	**−2.77 ± 0.22**	**−0.66 ± 0.19**
Adiposity				
Weight, kg	90.01 ± 0.31	90.28 ± 0.27	**−1.33 ± 0.04**	**−2.20 ± 0.07**
BMI, kg/m^2	31.97 ± 0.09	31.95 ± 0.08	**−0.47 ± 0.02**	**−0.79 ± 0.02**
GGT, U/l	37.74 ± 0.64	38.37 ± 0.58	**−3.89 ± 0.50**	**−4.34 ± 0.57**
Volume status and hematopoiesis				
Hematocrit, %	41.96 ± 0.06	42.00 ± 0.05	**2.34 ± 0.07**	**2.53 ± 0.05**
Hemoglobin, g/l	139.09 ± 0.22	139.54 ± 0.19	**6.63 ± 0.22**	**7.65 ± 0.18**
Serum albumin, g/l	41.40 ± 0.05	41.32 ± 0.04	**0.59 ± 0.04**	**0.56 ± 0.04**
Erythrocytes, $\times 10^{12}$ cells/l	4.68 ± 0.01	4.71 ± 0.01	**0.26 ± 0.01**	**0.27 ± 0.01**
Indicators of acidosis/alkalosis				
Serum bicarbonate, mmol/l	23.37 ± 0.04	23.33 ± 0.03	**−0.42 ± 0.05**	**−0.34 ± 0.03**
Other				
Serum urate, μmol/l	349.78 ± 1.47	348.21 ± 1.24	**−23.21 ± 1.16**	**−23.49 ± 1.02**

Values in **bold** indicate significant effect at $p < 0.05$. *Mixed-model repeated-measures analysis using all data available before completion in patients who had baseline and follow-up measurement for the respective outcome. The model adjusted for region, baseline HbA_{1c}, eGFR, BMI, baseline of the outcome, treatment, visit, and study subgroup (CANVAS or CANVAS-R). †Fasting test results. ‡Baseline data are geometric means (geometric coefficients of variation). §Differences are adjusted geometric mean ratio (95% CI) obtained from the mixed-model with repeated-measures analysis applied on log-transformed data.

BMI = body mass index; CI = confidence interval; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; FPG = fasting plasma glucose; GGT = gamma glutamyltransferase; HbA_{1c} = hemoglobin A_{1c}; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MDRD = Modification of Diet in Renal Disease; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride; UACR = urinary albumin:creatinine ratio.

history of symptomatic atherosclerotic cardiovascular disease or were 50 years of age or older with 2 or more risk factors for cardiovascular disease. Participants were required to have an estimated glomerular filtration rate (eGFR) at entry of more than 30 ml/min/1.73 m^2 of body surface area.

RANDOMIZATION AND STUDY TREATMENT. After a 2-week, single-blind, placebo run-in period, participants were randomized to canagliflozin or to matching placebo. Participants and all study staff were masked to individual treatment allocations until completion of the study.

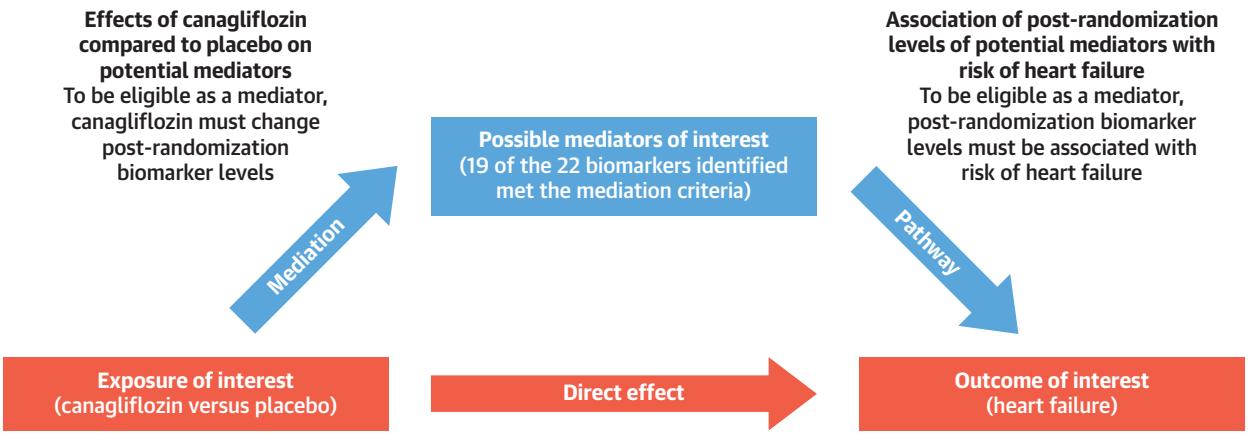
FOLLOW-UP. Participants were followed and randomized by face-to-face follow-up with 3 visits scheduled in the first year and further visits scheduled at 6-month intervals thereafter, with alternating telephone follow-up and face-to-face assessments. The occurrence of hospitalization for heart failure was evaluated at every scheduled follow-up.

OUTCOMES. The outcome studied in this analysis was the first hospitalized heart failure event during follow-up. An endpoint adjudication committee adjudicated all potential heart failure outcomes by using rigorous definitions that were pre-specified according to established criteria (9,10).

SELECTION OF POTENTIAL MEDIATORS. A diverse set of biomarkers was measured at baseline and at multiple time points during follow-up. The mediators considered for investigation in this analysis were identified through a process of consultation among the investigator group, with selection based upon insights derived from prior mediation analyses (8) and known mechanisms underpinning SGLT2 inhibition and heart failure. Biomarker choice was also dependent upon availability of data for assessment,

JACC: HEART FAILURE VOL. 8, NO. 1, 2020
JANUARY 2020:57–66

CENTRAL ILLUSTRATION **Selection of Possible Mediators and Criteria for Mediation**



Biomarkers considered as potential mediators (n = 22)
Glycemia **-** HbA_{1c}, FPG
Vascular tone **-** SBP, DBP, pulse rate
Lipids **-** LDL-C, HDL-C, TG, TC
Renal function **-** eGFR, UACR
Adiposity **-** weight, BMI, GGT
Volume status and hematopoiesis **-** hematocrit, hemoglobin, serum albumin, erythrocytes
Indicators of acidosis/alkalosis **-** urine pH, ketonuria, serum bicarbonate
Others **-** serum urate

Biomarkers meeting mediation criteria (n = 19)
Glycemia **-** HbA_{1c}, FPG
Vascular tone **-** SBP, DBP
Lipids **-** LDL-C, HDL-C, TC
Renal function **-** eGFR, UACR
Adiposity **-** weight, BMI, GGT
Volume status and hematopoiesis **-** hematocrit, hemoglobin, serum albumin, erythrocytes
Indicators of acidosis/alkalosis **-** urine pH, serum bicarbonate
Others **-** serum urate

Li, J. et al. J Am Coll Cardiol HF. 2020;8(1):57–66.

To be eligible as a mediator, it was necessary for there to be, first, an effect of canagliflozin compared to placebo on the potential mediator and, second, an association of post-randomization levels of the potential mediator with the risk of heart failure. A total of 22 biomarkers were considered potential mediators, and of these, 19 met the mediation criteria. BMI = body mass index; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; FPG = fasting plasma glucose; GGT = gamma glutamyltransferase; HbA_{1c} = hemoglobin A_{1c}; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride; UACR = urinary albumin:creatinine ratio.

and the absence of assays of B-type natriuretic peptide within the CANVAS Program dataset was noted as a significant shortcoming. Biomarkers that were believed unlikely to be changed by treatment with canagliflozin and unlikely to be associated with the risk of heart failure were excluded. Potential mediators were grouped into those likely acting through effects on glycemia, vascular tone, lipids, renal function, adiposity, volume status or hematopoiesis, acid-base balance, and serum urate (Table 1). FPG, urine pH, and ketonuria were measured in CANVAS but not in CANVAS-R (CANagliflozin cardioVascular Assessment Study-Renal; NCT01989754) and were assessed in subsidiary analyses restricted to CANVAS participants. Ketonuria was assessed as a dichotomous variable (none vs. trace or more), but all other potential mediators were assessed as continuous measurements.

STATISTICAL ANALYSIS. For the mediator to be eligible, it was necessary for there to be, first, an effect of canagliflozin compared to placebo on the potential mediator, and second, an association between post-randomization levels of the potential mediator and the risk of heart failure (Central Illustration). For the selection of potential mediators, the effects of canagliflozin versus placebo on the potential mediator were determined separately for each by using mixed models incorporating repeated measures of the variable of interest. The differences between groups were assessed by using residual restricted maximum likelihood tests. The exception to this approach was for the evaluation of ketonuria in CANVAS, which was assessed using a logistic regression model. Associations of the potential mediator with heart failure were determined from Cox regression models.

There were 2 ways by which the effects of the potential mediators were explored, reflecting prior work suggesting that both early (11) and later (12) effects of drugs on biomarkers may be important. Early change was determined by estimating, for every individual taking canagliflozin or placebo, the change in the potential mediator from baseline to the first post-randomization measurement, which was made at various time points between 6 and 18 weeks into follow-up. Average follow-up level was determined, for every individual taking canagliflozin or placebo, for all available measurements of the variable by using time-dependent analysis. Eligible measurements were those made at baseline and all measurements made prior to the first hospitalization for a heart failure event or prior to final follow-up for those who did not experience an event.

Variables with skewed distributions were analyzed after being log transformed (triglycerides [TG], UACR, and gamma glutamyltransferase [GGT]). FPG, urine pH, and ketonuria were available only from CANVAS. Individuals without a baseline measurement of the mediator of interest were excluded from the relevant analyses, as were individuals with no follow-up measurements and those with a baseline measurement who were hospitalized for heart failure before a follow-up measurement was made.

Primary analyses consisted of comparisons of HRs, from Cox survival models, for the association between randomized treatment and the risk of heart failure, unadjusted and adjusted for each biomarker, in turn. In each case, the percentage mediation was estimated by using the equation: $100\% \times \left(\frac{HR - HR_c}{HR - 1}\right)$, where HR_c is the hazard ratio after adjustment for the biomarker and HR is the unadjusted hazard ratio (13). The 95% CIs for the estimated percentage of mediation were obtained using a 10,000-iteration bootstrap resampling procedure. The combined potential mediating effect of multiple biomarkers was quantified using the same equation. Multiple mediator models were built by, first, selecting the biomarker with the largest percentage of mediation value. Each remaining biomarker was then included in turn, and the next biomarker that produced the greatest joint mediation was added to the existing model. This was repeated until the mediation effect reached 100% in the multivariable model. Only 1 variable from each biomarker group was included in the multivariable analysis because the goal was to capture different mechanistic processes likely to mediate the effects of the drug.

To further test the robustness of the findings, a secondary analysis was performed using the product method under the counterfactual framework approach (14) for univariable assessments and by using nonlinear models (multiple additive regression trees and smoothing splines) for multivariable assessments, which are able to account for the collinearity between potential mediators (15).

For every analytic approach evaluating the mediating effects of early change, the baseline level of the biomarkers was adjusted to control for regression to the mean. For the models assessing average effects, this was unnecessary because the baseline value already contributed to the calculation. All analyses were performed using SAS version 9.4 (Cary, North Carolina) and R studio version 1.1.463 (R Project, Vienna, Austria). The p values < 0.05 were deemed significant.

RESULTS

The potential mediating effects of 22 biomarkers were assessed, 19 of which were available for the entire CANVAS Program and 3 of which were available for participants in CANVAS but not CANVAS-R. The time to first measurement for the assessment of early biomarker changes was ≤13 weeks for 12 biomarkers, 12 to 26 weeks for 5 biomarkers, and 18 to 52 weeks for 5 biomarkers. For the assessments of average biomarker levels, the mean number of measurements made was least for hematocrit, hemoglobin, erythrocytes, urine pH, and ketones (mean 8 measurement times during follow-up) and most for blood pressure, pulse rate, weight, and body mass index (BMI) (mean 19 measurements during follow-up). The overall average number of biomarker measurements was 14.

EFFECTS OF CANAGLIFLOZIN COMPARED TO PLACEBO ON POTENTIAL MEDIATORS. There were clear effects of canagliflozin compared to placebo on multiple potential mediators of effect (**Central Illustration, Table 1**, Online Table 1). For example, hemoglobin A_{1c} (HbA_{1c}), FPG, systolic blood pressure (SBP), diastolic blood pressure (DBP), weight, BMI, urine pH, serum bicarbonate, serum urate, serum GGT, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), TG, hematocrit, hemoglobin, erythrocyte concentration, serum albumin, ketonuria, UACR, and eGFR.

ASSOCIATIONS OF POST-RANDOMIZATION LEVELS OF POTENTIAL MEDIATORS WITH RISK OF HEART FAILURE. For 14 of the 19 potential mediators (not SBP, pulse rate, HDL-C, TG, or eGFR) assessed in the overall CANVAS Program, there was a significant association between the early change in levels and the risk of heart failure in the regression models ($p < 0.05$)

TABLE 2 Observational Associations With Hospitalized Heart Failure of Potential Mediators of the Effects of Canagliflozin When Represented as Changes Measured Early After Randomization and as Average Levels During Follow-Up

	Early Change*		Average Level*	
	Hazard Ratio (95% CI)	p Value	Hazard Ratio (95% CI)	p Value
Glycemia				
HbA_{1c}	**1.23 (1.06–1.42)**	**0.007**	1.08 (0.97–1.20)	0.161
Vascular tone				
SBP	1.00 (0.99–1.01)	0.799	**1.01 (1.01–1.02)**	**0.002**
DBP	**0.98 (0.96–0.99)**	**0.009**	**0.98 (0.97–0.99)**	**0.004**
Pulse rate	1.00 (0.98–1.02)	0.879	**1.02 (1.01–1.03)**	**<0.001**
Lipids				
LDL-C	**0.79 (0.63–0.99)**	**0.037**	**0.78 (0.67–0.91)**	**0.002**
HDL-C	0.60 (0.26–1.37)	0.225	**0.41 (0.26–0.66)**	**<0.001**
TG	0.75 (0.50–1.13)	0.169	0.99 (0.76–1.29)	0.951
TC	**0.80 (0.66–0.96)**	**0.019**	**0.82 (0.72–0.93)**	**0.002**
Renal				
UACR	**1.36 (1.14–1.61)**	**0.001**	**1.50 (1.40–1.61)**	**<0.001**
eGFR (MDRD)	0.99 (0.97–1.00)	0.056	**0.98 (0.97–0.98)**	**<0.001**
Adiposity				
Weight	**1.08 (1.02–1.14)**	**0.009**	**1.02 (1.02–1.03)**	**<0.001**
BMI	**1.26 (1.06–1.48)**	**0.007**	**1.09 (1.07–1.11)**	**<0.001**
GGT	**1.60 (1.07–2.38)**	**0.022**	**1.95 (1.68–2.27)**	**<0.001**
Volume status and hematopoiesis				
Hematocrit	**0.93 (0.88–0.97)**	**0.001**	**0.94 (0.91–0.97)**	**<0.001**
Hemoglobin	**0.97 (0.96–0.99)**	**<0.001**	**0.98 (0.97–0.99)**	**<0.001**
Albumin	**0.92 (0.87–0.98)**	**0.006**	**0.82 (0.79–0.84)**	**<0.001**
Erythrocytes	**0.58 (0.37–0.92)**	**0.020**	**0.57 (0.43–0.75)**	**<0.001**
Indicators of acidosis/alkalosis				
Serum bicarbonate	**1.11 (1.05–1.18)**	**<0.001**	**1.10 (1.05–1.16)**	**<0.001**
Other				
Serum urate	**1.00 (1.00–1.01)**	**<0.001**	**1.01 (1.00–1.01)**	**<0.001**

Values in **bold** indicate significant effect at $p < 0.05$. *Values are the effect of a 1-unit post-randomization increase.

Abbreviations as in Table 1.

(Table 2). For the average post-randomization levels, there were significant associations observed for 17 of the 19 biomarkers (not HbA_{1c} or TG). The supplementary analyses of the biomarkers measured only in CANVAS identified additional significant associations between both the early change and average follow-up levels with the risk of heart failure for FPG and urine pH (Online Table 2).

ESTIMATED MEDIATION OF THE EFFECTS OF CANAGLIFLOZIN ON HEART FAILURE. There were 16 biomarkers (2 in CANVAS alone) for which early changes in post-randomization levels were achieved with canagliflozin versus placebo and for which those early changes in biomarker levels were also associated with the subsequent risk of heart failure (all except for SBP, pulse rate, HDL-C, TG, eGFR, and ketonuria). Just 3 of these 16 biomarkers (UACR, serum bicarbonate, and serum urate) were identified as individually statistically significant mediators of the effect of canagliflozin on heart failure, when the early changes in biomarker levels were assessed in the primary models (Table 3, Online Table 3). Assessment of joint effects of mediators representing different modes of action included hemoglobin, serum urate, and HbA_{1c} as the set of 3 that produced the largest combined percentage of mediation with an estimated cumulative mediation of 110% (95% CI: -379 to 877) of the effects of canagliflozin on heart failure (Table 4).

There were 17 biomarkers for which average post-randomization levels were modified with canagliflozin versus placebo and for which those early changes in biomarker levels were also associated with the subsequent risk of heart failure (SBP, DBP, TC, LDL-C, HDL-C, UACR, eGFR, weight, BMI, hematocrit, hemoglobin, serum albumin, erythrocytes, serum bicarbonate, serum urate, GGT, and urine pH). Fourteen of these biomarkers were identified as individually significant mediators of the effect of canagliflozin on heart failure, when the average post-randomization levels were assessed in the primary models. The 3 biomarkers with the largest magnitude of mediating effect were erythrocyte concentration, hemoglobin, and serum urate concentration (Table 3). Assessment of joint effects in a multivariable model of the strongest 3 mediators representing different modes of action resulted in the inclusion of erythrocyte concentration, serum urate, and UACR and an estimated cumulative mediation of 102% (95% CI: 42 to 480) of the effects of canagliflozin on heart failure (Table 4). Estimates were not substantively different if alternative biomarkers from the volume and hematopoiesis group (erythrocyte concentration, hematocrit, or serum albumin) were substituted for hemoglobin concentration (Online Table 4).

Subsidiary analyses based upon the alternative counterfactual framework identified 11 mediators based upon early changes in biomarker levels and 13 mediators based upon average levels. In the multivariable model, assessment of the early changes in hemoglobin, serum urate, and HbA_{1c} provided a value for the estimated overall mediation of 75% (95% CI: 47 to 101). For the corresponding model assessing average levels of biomarkers the inclusion of erythrocyte concentration, serum urate and UACR resulted in an estimated overall mediation of 94% (95% CI: 85 to 103).

DISCUSSION

The key methods and findings of this study are summarized in Table 5. A large set of potential

TABLE 3 Individual Assessments of Potential Mediators of the Effect of Canagliflozin on Heart Failure When Represented as Changes Measured Early After Randomization and as Average Levels During Follow-Up

	Early Change			Average Level		
	Events/Patients	Hazard Ratio (95% CI)	% Mediation (95% CI)	Events/Patients	Hazard Ratio (95% CI)	% Mediation (95% CI)
Unadjusted Hazard Ratio		**0.67 (0.52–0.87)**			**0.67 (0.52–0.87)**	
Adjusted for glycemia						
HbA_{1c}	218/9,854	0.77 (0.57 to 1.03)	23.0 (−8.30 to 111)	-	-	-
Adjusted for vascular tone						
SBP	-	-	-	223/9,988	0.75 (0.57 to 0.98)	11.8 (3.23 to 41.3)*
DBP	223/9,985	0.68 (0.52 to 0.89)	−12.6 (−61.0 to −2.55)	223/9,988	0.70 (0.53 to 0.91)	−7.21 (−23.1 to −2.52)†
Pulse rate	-	-	-	-	-	-
Adjusted for lipids						
LDL-C	198/9,436	0.75 (0.56 to 0.99)	4.39 (−2.86 to 31.1)	202/9,671	0.75 (0.56 to 0.99)	6.02 (1.91 to 26.2)*
HDL-C	-	-	-	202/9,674	0.76 (0.57 to 1.00)	9.29 (3.56 to 40.8)*
Triglycerides	-	-	-	-	-	-
TC	198/9,440	0.75 (0.56 to 1.00)	5.26 (−1.98 to 38.6)	202/9,675	0.75 (0.57 to 1.00)	7.65 (2.13 to 33.0)*
Adjusted for renal effects						
UACR	204/9,533	0.76 (0.58 to 1.01)	20.4 (3.74 to 93.7)*	206/9,637	0.79 (0.60 to 1.05)	29.8 (13.5 to 100)*
eGFR (MDRD)	-	-	-	223/9,975	0.71 (0.54 to 0.92)	−3.06 (−14.1 to 1.17)
Adjusted for adiposity						
Weight	223/9,977	0.77 (0.58 to 1.02)	18.9 (−14.0 to 133)	223/9,983	0.75 (0.57 to 0.98)	11.2 (4.81 to 35.2)*
BMI	222/9,964	0.77 (0.58 to 1.02)	21.0 (−11.3 to 118)	222/9,970	0.76 (0.58 to 1.00)	17.8 (8.29 to 53.1)*
GGT	223/9,967	0.75 (0.57 to 0.98)	11.7 (−2.43 to 59.9)	223/9,975	0.77 (0.59 to 1.01)	20.4 (10.0 to 63.0)*
Adjusted for volume status and hematopoiesis						
Hematocrit	170/9,014	0.85 (0.61 to 1.18)	45.7 (−12.5 to 320)	173/9,424	0.83 (0.60 to 1.13)	39.6 (9.86 to 153)*
Hemoglobin	173/9,106	0.88 (0.64 to 1.21)	52.1 (−150 to 449)	176/9,477	0.85 (0.63 to 1.16)	43.4 (12.4 to 206)*
Serum albumin	223/9,967	0.74 (0.57 to 0.97)	9.84 (−9.10 to 58.9)	223/9,975	0.82 (0.63 to 1.07)	36.2 (17.9 to 112)*
Erythrocytes	173/9,106	0.85 (0.61 to 1.18)	39.4 (−101 to 362)	176/9,477	0.86 (0.63 to 1.17)	45.1 (10.9 to 226)*
Adjusted for acidosis/alkalosis						
Serum bicarbonate	223/9,964	0.74 (0.57 to 0.97)	9.49 (1.16 to 43.1)*	223/9,972	0.74 (0.56 to 0.96)	7.06 (2.83 to 23.1)*
Adjusted for others						
Serum urate	223/9,967	0.79 (0.60 to 1.04)	27.0 (6.61 to 128)*	223/9,975	0.83 (0.63 to 1.08)	39.7 (20.5 to 122)*

Ten thousand bootstrap iterations were used to calculate 95% CI for percent mediation. *Potential mediators based on significant ($p < 0.05$) effects of canagliflozin on the biomarker and significant ($p < 0.05$) associations of the biomarker with the future risk of heart failure, together with evidence of significant mediation. †DBP was shown to have a negative mediating effect on heart failure.

Abbreviations as in Table 1.

mediators of the effect of canagliflozin on heart failure was identified. Some of the mediators, such as markers of plasma volume, are highly plausible based upon the known causes of heart failure (16). Others, such as SBP, were not identified as strong or consistent mediators despite long-established effects of blood pressure–lowering agents on heart failure and clear effects of canagliflozin on blood pressure (17). Others still, such as blood lipid levels, were identified as weak mediators but with no known mechanism of action. The most consistent finding across all the analyses was of strong mediating effects for the markers of volume and hematopoiesis and for mediating effects of uric acid. Mediating effects for albuminuria in many of the models highlights a likely central role for the cardiorenal axis in mediating the effects of canagliflozin on heart failure.

Volume reduction would be anticipated to mediate the prevention of heart failure primarily by reducing preload (16). Observed mediating effects of adiposity are also likely, partly at least because they reflect volume effects with early reductions in weight, probably due to fluid loss, whereas later incremental weight loss probably indicates reduction in fat mass (5). Effects of canagliflozin on GGT were included as an indicator of adiposity effects because changes in GGT are likely to be due to reductions in hepatic steatosis (18,19).

In addition to signaling diuresis and a decrease in plasma volume, effects of SGLT2 inhibition on serum hemoglobin, erythrocytes, hematocrit, and serum albumin could also indicate changes in red cell mass due to an effect on erythropoiesis (20). Dapagliflozin therapy has been reported to raise levels of erythropoietin soon after it is initiated, with increases in reticulocyte count noted prior to subsequent elevation of hemoglobin and hematocrit (21). Enhanced delivery of oxygen to the tissues has been postulated as a mechanism for the benefits of SGLT2 inhibition

TABLE 4 Joint Assessments of Potential Mediators of the Effect of Canagliflozin on Heart Failure When Fitted as Changes Measured Early After Randomization and as Average Levels During Follow-Up

	Early Change				Average Level		
	Events/Patients	Hazard Ratio (95% CI)	% Mediation (95% CI)		Events/Patients	Hazard Ratio (95% CI)	% Mediation (95% CI)
Unadjusted Hazard Ratio		**0.67 (0.52–0.87)**				**0.67 (0.52–0.87)**	
Adjusted for							
Hemoglobin	173/9,106	0.88 (0.64 to 1.21)	52.1 (−150 to 449)	Erythrocytes	176/9,477	0.86 (0.63 to 1.17)	45.1 (10.9 to 226.0)
+ Serum urate	173/9,104	0.97 (0.71 to 1.34)	89.4 (−358 to 733)	+ Serum urate	176/9,475	0.96 (0.70 to 1.31)	84.6 (36.5 to 356.0)
+ HbA_{1c}	173/9,104	1.03 (0.73 to 1.45)	110 (−379 to 877)	+ UACR	173/9,264	1.01 (0.73 to 1.38)	102 (42.1 to 480.0)

Abbreviations as in Table 1.

on kidney disease (20), and parallel mechanisms may also avert hypoxia in the heart (22).

Reduction in albuminuria with SGLT2 inhibition is believed to be jointly attributable to changes in the glomerular filtering of albumin and increased tubular reabsorption. Favorable effects on endothelial function, which improve endothelial glycocalyx barrier function in the kidney, have been postulated, and parallel benefits in other vascular beds may explain improvements in cardiac function (23,24).

The lowering of uric acid achieved with SGLT2 inhibitor treatment is believed to result from enhanced urinary excretion (25). Elevated serum urate levels have been associated with heart failure and other vascular diseases in prior studies (26), but there is no clear understanding of how uric acid lowering due to SGLT2 inhibition would drive reduction in heart failure risk.

Little evidence was found for effects on glycemia mediating the impact of canagliflozin on heart failure, although the only moderate effects of canagliflozin on glycemia and the absence of systematic assessments of plasma glucose during follow-up may have mitigated against the detection of effects.

It has been hypothesized that, under persistent mild ketosis caused by SGLT2 inhibition (27), there may be preferential uptake of β-hydroxybutyrate by the myocardium and protection of the failing heart (28). Imprecise measurement of ketonuria and data collection restricted to the CANVAS trial alone might have mitigated against detection of a mediating effect of ketosis. The observed reduction in bicarbonate and associated mediating effect may be due to effects of canagliflozin on sodium hydrogen exchanger-3 (NHE3) (16,29). Inhibition of SGLT2 down-regulates NHE3 and may mediate effects of canagliflozin on heart failure through a natriuretic effect (21).

SBP was identified as a weak and inconsistent mediator of the effect of canagliflozin on heart failure in these analyses, and DBP was consistently shown to have a moderate-sized negative mediating effect. There is no clear explanation for this latter observation, but the failure to detect a mediating effect for SBP may be a consequence of the substantial within-person variability of SBP, which reduces the capacity to precisely define effects on SBP at an individual level.

The EMPA-REG OUTCOME trial has previously sought to understand the mechanism through which empagliflozin protected against death from cardiovascular disease. Those analyses concluded that effects on plasma volume were the most important mediators of effect but also highlighted the role of changes in FPG, UACR, and uric acid (8). Although CIs for estimates of mediating effects were not provided in that report, there does appear to be some commonality of findings, albeit for a different clinical outcome.

STUDY LIMITATIONS. These analyses of the CANVAS Program benefited from the large size of the dataset, the high quality of trial conduct, the range of biomarkers available for analysis, the robust adjudication of heart failure outcomes, and the range of methods applied to assess mediation. There were also multiple measurements available

TABLE 5 Key Methods and Findings

Canagliflozin reduced the risk of heart failure among patients with type 2 diabetes in the CANVAS (CANagliflozin cardioVascular Assessment Study) Program. The mechanism of protection was explored by fitting univariable and multivariable models to assess mediation.

- Biomarkers were selected for investigation based upon availability and agreement among the investigator team members.
- Assessment for mediation was done only for biomarkers that were changed by canagliflozin and associated with heart failure.
- Markers of volume were the strongest mediators of risk followed by serum urate and urinary albumin:creatinine ratio.
- Subsidiary analyses were broadly consistent in the findings.

Identified mediators support existing and novel hypothesized mechanisms for the prevention of heart failure with sodium glucose cotransporter 2 (SGLT2) inhibitors.

for most potential mediators, and there was no need for imputation of data. However, there were also important limitations. All these investigations were performed post hoc and required multiple statistical tests, and the results are hypothesis-generating in every case. Only the potential mediating effects of biomarkers measured during the trial were able to be assessed, and it was not possible to directly assess the potential role of B-type natriuretic peptide and pathways acting through mechanisms such as inflammation (30), oxidative stress (31), arterial stiffness, or vascular resistance (32). Also, the authors were unable to adjust for potential effects of competing risks. There are significant challenges inherent in the statistics underlying the methodologies with limited capacity to control for interactions between mediators and provide robust estimates of uncertainty. The findings were sensitive to whether the mediator was explored in terms of the early effect or the average effect, which may reflect the capacity of the different methodologies to detect mediating effects, as much as it does real differences in early versus late mechanistic pathways. For example, assessment of mediation based upon early changes after follow-up are likely to be systematically underestimated compared to assessment of mediation based upon average changes, because statistical estimates based upon 1 or 2 measurements of a biomarker are much less powerful than those based upon multiple measures. Assessments of the joint effects of mediators resulted in more than 100% of the effect explained with only 3 mediators included, and this highlights the limited capacity to explore and control for double-counting of a mechanistic pathway captured by more than 1 biomarker. Finally, it is not possible to be sure that the effects identified are truly part of the mechanistic pathway for heart failure prevention by canagliflozin rather than an epiphenomenon associated with both the effects of canagliflozin and the future risk of heart failure.

CONCLUSIONS

We identified a diverse set of potential mediators of the effect of canagliflozin on heart failure. Some mediating effects were anticipated, and others were not. These analyses provide support for most of the previously hypothesized mechanisms for the prevention of heart failure with SGLT2 inhibitors, but the extent to which each marker truly mediates the beneficial effect of canagliflozin on heart failure remains uncertain.

ACKNOWLEDGMENTS Findings are reported on behalf of the CANVAS Program collaborative group. The authors thank all investigators, study teams, and patients for participating in these studies. Editorial support was provided by Alaina Mitsch, PhD, MedErgy, and was funded by Janssen Global Services, LLC. Canagliflozin was developed by Janssen Research & Development, LLC, in collaboration with Mitsubishi Tanabe Pharma Corporation.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Sodium glucose cotransporter 2 (SGLT2) inhibitors provide significant protection against heart failure. This benefit was not anticipated when the trials of these agents were initially designed. Mediation analyses can provide insights into the mechanism by which drug effects are achieved and may enable the better targeting of therapy to patient groups most likely to benefit.

TRANSLATIONAL OUTLOOK 1: The statistical tools upon which mediation analyses are based have significant limitations, even when applied to very large datasets. Applying a breadth of analytic approaches emphasizes the extent to which findings depend upon the methodology selected and highlights the level of persisting uncertainty.

TRANSLATIONAL OUTLOOK 2: Smaller, dedicated mechanistic studies specifically designed to test the mediating effects of individual candidate biomarkers may be a more effective way of defining the mechanism by which SGLT2 inhibition prevents heart failure.

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KEY WORDS canagliflozin, heart failure, mediation analysis, renal outcomes

APPENDIX For supplemental tables, please see the online version of this paper.